

## SHORT REPORT

## Mild recurrent neuropathy in CMT1B with a novel nonsense mutation in the extracellular domain of the MPZ gene

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### Abstract

**Clinical, electrophysiological, and neuropathological features are reported associated with a novel heterozygote point mutation in the extracellular domain of the MPZ gene, where a transversion at codon 71 in exon 3 leads to a codon stop: Glu71stop (ie GAA→TAA). A 36 year old woman developed a mild recurrent neuropathy after intensive manual work. The motor nerve conduction velocities were slow without conduction blocks and the nerve biopsy showed signs of demyelination-remyelination, axonal loss, and regular uncompacted myelin lamellae. She inherited the mutation from her father who displayed the same mutation with a normal phenotype. This nonsense mutation may cause a dosage difference of normal P0, and is probably underrepresented in the current mutation data bases. This report further extends the phenotype of MPZ mutations and also emphasises that mild phenotype of CMT1B may be more frequent than has been appreciated.** (*J Neurol Neurosurg Psychiatry* 2001;70:232-235)

Keywords: MPZ gene; Charcot-Marie-Tooth type1B; nonsense mutation

The human gene encoding P0 glycoprotein (MPZ) is located on chromosome 1q21-q22 and comprises six coding exons.<sup>1</sup> MPZ, expressed exclusively in myelinating Schwann cells, is located in compact myelin and is the most abundant structural protein of peripheral myelin sheaths.<sup>2</sup> It contains three domains, a large glycosylated immunoglobulin-like extracellular domain, a transmembrane domain, and an intracellular domain, each one playing an essential part in the formation and the maintenance of the myelin.<sup>3</sup> About 80 different mutations have been reported in the MPZ gene, most concerning the extracellular domain which exhibits homotypic properties, which are essential in the compaction of the intraperiod lines of myelin sheaths.<sup>4</sup> Mutations in MPZ usually result in Charcot-Marie-Tooth disease (CMT1B).<sup>5</sup> However, the range of the phenotypes is very wide,<sup>6-9</sup> and correlations between the phenotypes and the individual

mutations are useful. Here we report clinical, electrophysiological, and pathological findings in a 36 year old woman carrying a novel heterozygous point mutation in the extracellular domain, where a transversion at codon 071 in exon 3 leads to a stop codon: Glu 71 stop (GAA→TAA).

### Case report

A 36 year old woman (family pedigree II:1) was referred for sensory disturbances which had appeared 6 months earlier during a 1 month seasonal period of work in a vineyard. The first symptoms were a burning sensation on the soles and paraesthesia with numbness in the right hand. Progressively these sensory disturbances extended to the lower legs and the left hand, the paraesthesia in the right hand remaining the most troublesome. She mentioned that disturbances had appeared each year since she had started seasonal work 4 years ago. The sensory disturbances were sometimes accompanied by transient weakness in the legs, usually following prolonged squatting positions and lasting a few minutes. However, during the first 3 years, she had not sought medical advice because the disturbances disappeared in less than a month after the end of her seasonal work. She had run several half marathons up to the age of 27 without weakness, pain, or cramps in the lower limbs.

At examination there was no muscle atrophy. She was able to walk on tiptoes and heels and to stand up from the squatting position without difficulty. Motor power analysis showed no weakness, in particular in the limb extremities. Pinprick sensation was diminished in a stocking distribution in the lower limbs and in the sensory area of the right median nerve was associated with Tinel's sign. Vibration sensation was impaired distally in the legs on both sides, but the joint position was preserved and there was no Romberg's sign. Achilles reflexes were abolished; the other tendinous reflexes were present and the plantar responses were in flexion. She had a bilateral pes cavus, but the rest of the clinical examination was normal. No familial history of neuropathy was known.

Motor nerve conduction velocities (MNCVs) were slow (table) without conduction blocks. Terminal latency indices (TILs),

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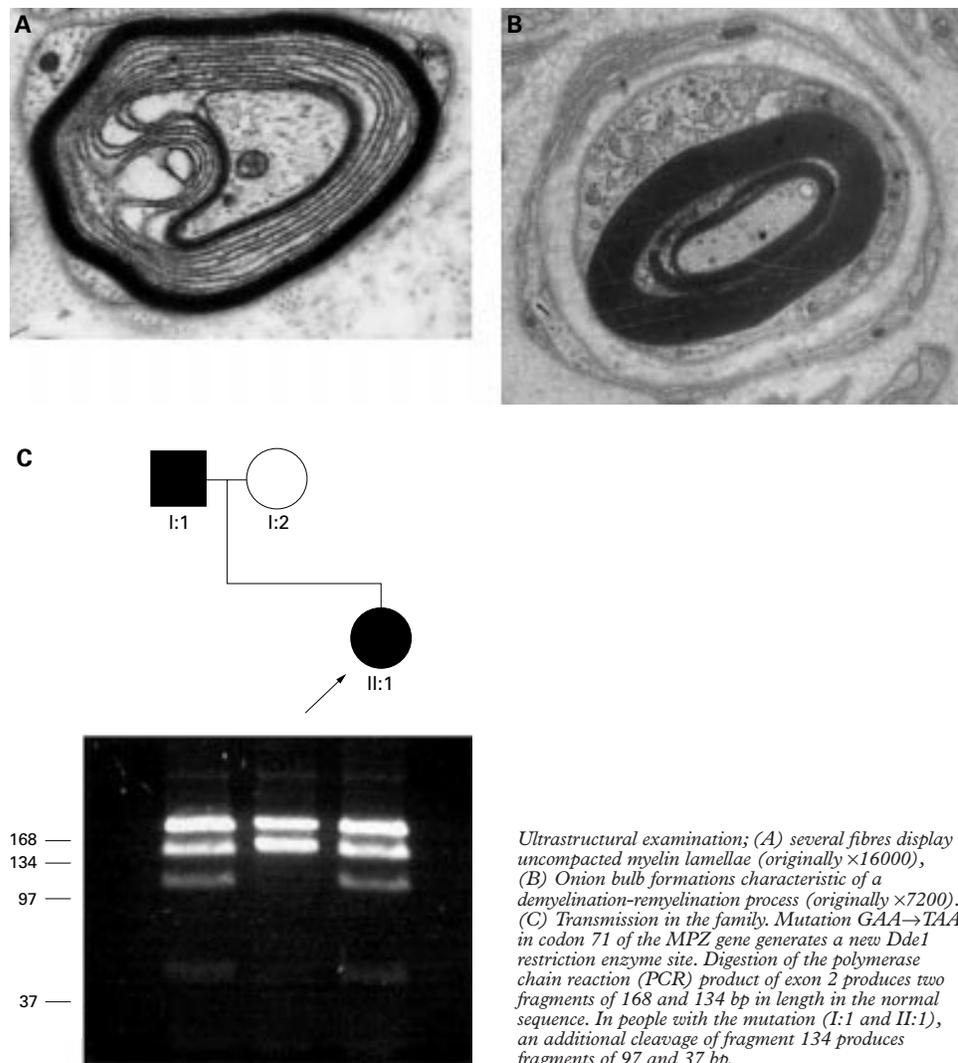
Table 1 Electrophysiological results

Nerves	Segments	MNCV (m/s)	CMAP amplitude (mV)	DL (ms)	TLI (normal $\geq 0.34$ )	SNCV (m/s)	SNAP amplitude ( $\mu V$ )
Left peroneal	Leg	31.8	2.9	5.9	0.39		
	Knee	38.8	1.9				
Right peroneal	Leg	33.8	3.6	5.2	0.43		
	Knee	28	2.9				
Left median	Forearm	34.2	6.0	3.9	0.37		
	Palm wrist						43
Right median	Forearm	31	7.0	5.8	0.27		
	Palm wrist						40
Left ulnar	Forearm	43.8	6.1	2.9	0.39		
	Elbow	43.3	6.1				
Right ulnar	Palm wrist					41.3	17.7
	Forearm	40.2	7.2	3.5	0.49		
	Elbow	52.6	6.0				
Right sural	Palm wrist					43.3	15.5
Left sural	Lower leg-ankle					ND	0
	Lower leg-ankle					ND	0

ND=not determinable; average (SD) control values: MNCV, median=55 (4.5), DL=3.8 (0.4), CMAP amplitude=12 (2.4); ulnar=54.6 (3.6), DL=2.6 (0.3), CMAP amplitude=9.5 (2.5); peroneal : 49 (5.5), DL=4.5 (0.6), CMAP amplitude=6.3 (1.6); SNCV, median=52 (3.5); SNAP amplitude=12.5 (3.4); sural=48 (4); SNAP amplitude=16 (4.5).

calculated as follows: terminal distance/(distal motor latency (DL) $\times$ proximal conduction velocity), were in the normal range except in the right median nerve, where they were higher. Compound muscle action potential (CMAP) amplitudes were low in the lower limbs and normal in the upper limbs. Orthodromic sensory nerve conduction velocities (SNCVs) were bilaterally slow in the median and the

ulnar nerves between the palm and wrist (table). The sensory nerve action potential (SNAP) amplitude from the right median nerve was diminished by 50% compared with the left, a finding in association with an abnormal TIL, which was consistent with the diagnosis of a right carpal tunnel syndrome. The SNAPs from the sural nerves were undetectable at the ankle after stimulation at



Ultrastructural examination; (A) several fibres display uncompact myelin lamellae (originally  $\times 16000$ ), (B) Onion bulb formations characteristic of a demyelination-remyelination process (originally  $\times 7200$ ). (C) Transmission in the family. Mutation GAA $\rightarrow$ TAA in codon 71 of the MPZ gene generates a new DdeI restriction enzyme site. Digestion of the polymerase chain reaction (PCR) product of exon 2 produces two fragments of 168 and 134 bp in length in the normal sequence. In people with the mutation (I:1 and II:1), an additional cleavage of fragment 134 produces fragments of 97 and 37 bp.

the lower third of the legs. The needle examination showed signs of moderate partial denervation in the two extensor hallucis at full effort without activities at rest. Latencies of the P100 (VEPs) and of the peaks from I to V of the brain stem evoked auditory responses were bilaterally normal. Routine laboratory tests of serum and CSF were normal.

A nerve biopsy of the superficial peroneal nerve was performed. No abnormality was seen at light examination of paraffin embedded sections. A quantitative study was performed from paraphenylenediamine stained transverse semithin sections using a semiautomatic analyser. This study evidenced a marked loss of myelinated fibres (5538/mm<sup>2</sup>; lower limit of normal 7000/mm<sup>2</sup>), with a bimodal distribution. Some lipid laden macrophages and some onion bulb formations were also seen on semithin sections. Teased fibre studies were not carried out. Ultrastructural examination showed numerous clusters of regenerating myelinated fibres and numerous features of "collagen pockets" among unmyelinated fibres. Macrophagic histiocytes overloaded with myelin debris were seen in the vicinity of a remyelinating axon and "onion bulb formations" were surrounding remyelinating axons. Several fibres with regularly uncompacted myelin lamellae were seen (figure A).

A mutation analysis was performed. Duplication in 17p11.2 was absent and mutations in PMP22 were excluded by sequencing. The MPZ gene was screened for the presence of mutations as previously described.<sup>10</sup> Sequencing disclosed a heterozygous point mutation in the P0 extracellular domain, where a transversion at codon 71 in exon 3 leads to a codon stop: Glu71stop (GAA→TAA). Blood samples from both parents were screened for the presence of the mutation. Neither complained of symptoms, their neurological examination was normal, and they did not display pes cavus. The asymptomatic father (I:1), a 62 year old man, carried the same mutation (figure B). Unfortunately, he refused an electrophysiological examination.

## Discussion

Our patient presented with a mild recurrent sensory neuropathy. The electrophysiology was consistent with a diffuse demyelinating neuropathy associated with signs of axonal loss in the lower limbs. The symptoms recurred during each period of intensive manual work, which could suggest a hereditary neuropathy with liability to pressure palsy.<sup>11</sup> However, except for the electrophysiological signs of the right carpal tunnel syndrome, the conduction slowings did not prevail in the common sites of nerve compression and the TILs were normal (table). The nerve biopsy showed a process of demyelination-remyelination with onion bulb formations and some degree of axonal damage. Some fibres had regular uncompacted myelin lamellae. This pattern has been seen in CMT1B where two divergent patterns, uncompacted myelin on one hand and tomaculae on the other, have been found.<sup>12</sup> However, in

CMT1B, irregular decompaction seems to be more frequent,<sup>10,13</sup> than regular decompaction.<sup>14</sup>

Duplication in the PMP22 gene responsible for 70% of the CMT1 was absent. Detection of uncompaction of myelin lamellae on the ultrastructural examination, although not specific to CMT1B,<sup>15</sup> led to the screening of the MPZ gene. A novel heterozygous point mutation was found in P0ex at codon 71, with a mutation leading to a stop codon (GAA→TAA). This mutation was also found in the father, who was clinically asymptomatic, by restriction enzyme analysis (figure B). To our knowledge, this is the first nonsense mutation found in the P0 extracellular domain, where most of the mutations in MPZ occur. The prematurely terminated protein does not possess a transmembrane domain, which is essential for anchorage in the cell membrane. If this truncated protein is transported, it may get lost in the extracellular space. On the other hand, intracellular degradation could also occur.<sup>16</sup> Both mechanisms lead to dosage effects, and the limited number of functional P0 molecules may be responsible for the formation of the abnormal myelin found at ultrastructure. The mild phenotype suggests that the mutated protein produced may be too short to interfere with tetramer formation contrary to the severe phenotypes present in two mutations occurring at the extra cellular/transmembrane interface.<sup>16,17</sup>

In our patient a carpal tunnel syndrome occurring after intensive manual workings disclosed the neuropathy. It may be supposed that in heterozygotes, the occurrence of this type of mutation in the extracellular domain is under-represented in current mutation data bases (<http://molgen-www.uia.ac/CMTMutations/>). Other mutations in MPZ have been described before, where the heterozygous were clinically asymptomatic with mild slowing of NCVs, while the homozygous children had severe Dejerine-Sottas disease.<sup>18,19</sup> This pattern has been found in the experimental model described by Martini *et al*,<sup>20</sup> where heterozygous mice (P0-/+ ) have a normal phenotype whereas homozygous mice (P0-/-) develop an early severe phenotype. CMT1B is rare, but its prevalence is probably underestimated because mild phenotypes in heterozygotes may go unrecognised whereas a dosage effect could lead to a severe phenotype in homozygotes.

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